REMARKS/ARGUMENTS

Claims 1-4, 8-16, 20-32, 36-44 , and 48-68 are pending in the present application. In the Office Action, the Examiner objected to the amendments to the specification in the last amendment, because they allegedly add new matter to the specification. Claims 1, 8-13, 20-29, 36-41, 48-52 and 53, 57, 61, and 65 were rejected under 35 U.S.C. § 112, first paragraph, for allegedly lacking enablement. Claims 1-4, 8-10, and 12 were rejected under 35 U.S.C. §§ 102(b) and 102(e) for allegedly being anticipated by EP 0902035 A2 and US Patent No. 6,346,379, respectively. Claims 11, 13-16, 20-32, 36-44, 48-68 were rejected under 35 U.S.C. § 103(a), for allegedly being obvious over the above references in view of Kawasaki (PCR Protocols).

Objection to the Specification

The Examiner has objected to the amendment of the specification to incorporate the priority application, USSN 60/198, 336, by reference. In response, applicants have amended the specification to remove the incorporation by reference. Withdrawal of the objection is respectfully requested.

Rejections under 35 U.S.C. § 112, first paragraph

In the Office Action, at page 3, the Examiner maintains the rejection of the claims for allegedly lacking enablement. In the Office Action, the Examiner alleges that making and testing mutants within the scope of the rejected claims would require undue experimentation.

As explained in the previous response and in MPEP § 2164.04, the Examiner has the initial burden to establish a reasonable basis to question the enablement provided for the claimed invention. *In re Wright*, 999 F.2d 1557, 1562, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993). As stated by the courts, "it is incumbent upon the Patent Office, whenever a rejection on this basis is made, to explain *why* it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement. Otherwise, there would be no need for the applicant to go to the trouble and expense of supporting his presumptively accurate disclosure." *In re Marzocchi*, 439 F.2d 220, 224, 169 USPQ 367, 370 (CCPA 1971).

In the response to Applicant's previous arguments, the Examiner acknowledges that "detecting the requisite motif and the introduction of single amino acid substitutions are within the skill of the art." The Examiner asserts, however, that the effect of single amino acid substitutions on the activity of the enzyme is "unpredictable" (*see* Office Action, page 6, first full paragraph).

The Examiner's concern apparently lies in the fact that the rejected claims define four residues in an eleven residue motif (*see* Office Action, page 5, lines 18-19). In particular, the rejection appears to be based on the assertion that one of skill must make and test over 4 billion sequences to identify enzymes having the claimed activity (*see* Office Action page 6, line 4). On page 7, first full paragraph, the Examiner further asserts that the patent application fails to disclose the primary structure of DNA polymerase enzymes that have reverse transcriptase activity. Applicants are unclear as to the basis for this assertion since the specification provides very clear disclosure of mutations useful in conferring this activity on DNA polymerases. If the Examiner's concern relates, instead, to the identification of thermoactive DNA polymerases in their native form, this concern is also misplaced, as explained in detail below.

The mutated enzymes used in the claimed methods are those that, "in their native form," comprise a motif defined by SEQ ID NO: 1. The term "native" is defined on page 7, lines 23-26, to refer to gene products which are isolated from nature or recombinant forms that have the same amino acid sequence as the naturally occurring form. Thus, the claims are directed to methods in which the starting enzymes from which the mutant enzymes are derived are naturally occurring.

The attached declaration of Dr. David H. Gelfand provides evidence that the structure and function of thermoactive DNA polymerases were well understood at the time of the invention (*see* Gelfand Declaration, ¶ 4). Because of their use in PCR amplification assays and for DNA sequencing, thermoactive DNA polymerases had been the subject of study for over fifteen years at the time the present application was filed. At the time of filing, dozens of eubacterial thermoactive or thermostable DNA polymerase enzymes had been identified. Indeed, many high-resolution crystal structures of DNA polymerases derived from thermophilic microorganisms have been published.

Based on these high resolution crystal structures, the structural features of DNA polymerases derived from thermophilic microorganisms were well known. As explained by Dr. Gelfand, position 4 of the claimed motif is thought to be important to the reverse transcriptase activity of the enzyme because mutations at position 4 are likely to result in "tighter binding" of said polymerase to primer-template substrate (*see* Gelfand Declaration, ¶ 5).

Moreover, Dr. Gelfand, shows that the steps in the identification, making and testing of enzymes was entirely routine using the teachings of the present application. Based on these known structural features, using standard sequence alignment programs described, for example, at page 13, lines 18-28 of the present application, one of skill could readily identify candidate thermoactive DNA polymerases. If necessary, one of ordinary skill in the art could easily verify whether a particular enzyme has thermoactive DNA polymerization activity using simple experiments well-known to the art. For example, the ordinarily skilled artisan could identify that an enzyme has DNA polymerization activity by performing a primer extension assay. The thermostability of the enzyme can easily be tested by heating the enzyme before the assay. Alternatively, the ordinarily-skilled artisan could simply refer to the extensive literature to identify a suitable candidate enzyme for use in the methods of the invention (*see* Gelfand Declaration ¶6).

As explained by Dr. Gelfand, the Examiner's concern that an unwieldy number of enzymes would be generated is misplaced. To prepare such enzymes, one of skill would not synthesize and test 4 billion enzymes containing each of the species of motif as implied by the assertions at the top of page 6 of the Office Action. Rather, one of skill in the art would determine whether a previously identified thermoactive DNA polymerase comprises the motif defined by SEQ ID NO: 1 using the alignment algorithms noted above. Such sequence comparisons are entirely routine in the art (*see* Gelfand Declaration, ¶ 7). As noted by Dr. Gelfand and demonstrated in the specification, the applicants have identified thermoactive DNA polymerase enzymes from 12 different bacterial species that comprise the motif. See Table 1 at page 12, lines 1-20.

In the Office Action at page 3, the Examiner explicitly acknowledges that making and testing mutants of an exemplified DNA polymerase (*Thermus thermophilus*, which

comprises SEQ ID NO: 3) is enabled by the present specification. In addition, the Examiner has not rejected claims directed to use of mutants of SEQ ID NOs: 2-7 (claims 2-4, 14-16, 30-32, 42-44, 62-64, and 66-68). Thus, applicants understand that the Examiner acknowledges that one of skill can make and use variants of enzymes encompassed by these claims without undue experimentation. The Examiner apparently asserts that the generation of similar mutants from other DNA polymerases would require undue experimentation.

As explained by Dr. Gelfand in paragraph 8, after identifying a particular thermostable DNA polymerase that comprises the claimed motif, one of skill then determines whether the thermostable DNA polymerase enzyme naturally comprises an appropriate residue at position 4 of the motif. If the thermostable DNA polymerase enzyme does not naturally comprise the appropriate residue at position 4 of the critical motif, the ordinarily-skilled artisan can routinely construct such a polymerase using, for example, site directed mutagenesis protocols as described in the specification at page 14, lines 23-27. If the thermostable DNA polymerase enzyme naturally comprises an appropriate residue at position 4 of the critical motif, the ordinarily-skilled artisan will recognize that the thermostable DNA polymerase enzyme is suitable for use in the methods of the present invention without further alteration. Thus, one of ordinarily skill in the art can make thermostable DNA polymerases for use in the methods of the invention with no more than routine experimentation.

The Examiner is apparently concerned that because amino acid residues comprising the critical motif are not completely conserved among all DNA polymerases, the effect of changes to the amino acid residues other than position 4 would be unpredictable. As explained by Dr. Gelfand, one of skill would recognize that the lack of complete conservation within the claimed motif in thermoactive DNA polymerases is not critical to practicing the invention. As explained above, thermoactive DNA polymerases are well-characterized. The motifs and domains discussed above provide more than sufficient guidance as to which residues, if any, can be mutated for desired properties in the final enzyme. Moreover, the effect of any particular mutation can be readily tested in routine assays (*see* Gelfand Declaration ¶9). Moreover, Dr. Gelfand and his colleagues have prepared and tested three other Designer DNA polymerases in addition to the enzymes exemplified in the specification. As can be seen by the

results described in his declaration, despite variability in positions inside and/or outside the motif identified here, reverse transcriptase activity is enhanced by mutation at position 4 of the claimed motif.

In paragraphs 11-16, Dr. Gelfand provides evidence from two Designer DNA Polymerases (ES112 and ES113). ES112 is the E683R mutant form of *Thermus* specie Z05 DNA Polymerase (SEQ ID NO: 11). ES113 is the E683K mutant form of *Thermus* specie Z05 DNA Polymerase (SEQ ID NO: 11). In both of these DNA polymerases the "X" residue at position 4 of SEQ ID NO: 1, or the "E" residue at position 4 in SEQ ID NO: 2 and SEQ ID NO: 3 have been mutated as taught in the specification. The third enzyme, CS6 DNA Polymerase, is a chimeric Designer DNA Polymerase comprising the DNA polymerase domain of *Thermotoga maritima* DNA Polymerase (SEQ ID NO: 15). CS6 DNA Polymerase is more fully described in copending US Patent Application Serial No. 10/401,403, filed March 26, 2003 as SEQ ID NO:107 and in Fig. 5A. In CS6 DNA Polymerase, the "X" residue at position 4 of SEQ ID NO: 1 of the present specification is an arginine (R) and in SEQ ID NO: 5 the 4th residue is also arginine (R). All of these enzymes contain the "Critical Motif" as taught in Table 1 of the specification and all have improved reverse transcription (RT) capabilities.

Exhibit 1 ("Improved Mg^{2+}-activated RT-PCR with ES112 & ES113") of Dr. Gelfand's Declaration provides evidence that ES112 and ES113 are magnesium-activated thermoactive and thermostable reverse transcriptases as well as possessing manganese-activated thermoactive and thermostable reverse transcriptase activity (*see* Gelfand Declaration ¶12).

Exhibit 2("Reduced RT Time Requirement for ES112 & ES113 in Mn^{2+}") provides evidence that both the ES112 and ES113 mutant DNA polymerases are improved and are much "faster" reverse transcriptases than *Thermus* specie (wild-type) Z05 DNA Polymerase. The improved reverse transcriptase enzymes of the invention could contribute significantly to a shorter time requirement for RT-PCR screening assays (Gelfand Declaration ¶13).

Exhibit 3 ("Efficient RT-PCR at Decreased ES112 & ES113 Enzyme Concentrations") shows that a lower concentration of the improved reverse transcriptases is sufficient for efficient single enzyme RT and PCR (Gelfand Declaration ¶14).

Exhibit 4 ("Improved Low Copy Sensitivity with ES112 in Mn^{2+}-activated RT-

PCR") shows that the improved reverse transcriptases of the invention are characterized by improved reverse transcription efficiency and target detection sensitivity (Gelfand Declaration ¶15).

Exhibit 5 ("RT-PCR Using Mg^{2+}-activated CS6 DNA Polymerase") shows that Designer DNA Polymerase CS6, a *Thermotoga maritima*-derived DNA polymerase domain chimeric enzyme with a polymerase domain entirely unrelated to the *Thermus*-derived DNA polymerases described above, is also a magnesium-activated thermostable and thermoactive reverse transcriptase. As explained by Dr. Gelfand, the "Critical Motif" (Table 1 in the specification) shows that the majority of the amino acids (6 of the 11 positions) are different between many of the *Thermus* genus enzymes and the *Thermotoga* genus enzymes. Nevertheless, when the teachings of the specification are followed, improved reverse transcriptases are obtained. Finally, there is a great deal of amino acid sequence divergence between the *Thermus* genus DNA polymerases and the *Thermotoga* genus DNA polymerases, reflecting the considerable evolutionary divergence of the microorganisms from which the enzymes originally derive. Indeed, there is only about 45% overall amino acid identity between aligned DNA polymerase sequences from representatives of the *Thermus* genus and representatives of the *Thermotoga* genus. That is, more than half of the amino acids are different. However, SEQ ID NO:1 and the "Critical Motif" (Table 1) of the invention can readily be uniquely identified in the DNA polymerase domains of these microorganisms' DNA polymerases (Gelfand Declaration ¶16).

In light of this evidence, the Examiner must explain why mutation of DNA polymerases other than those exemplified or acknowledged to be enabled would present unusual problems in terms of testing for reverse transcriptase activity in light of the teaching in the present application. In the absence of such a showing, Applicants submit that testing other DNA polymerases enzymes that can be identified using SEQ ID NO: 1 is entirely routine, despite the large number of enzymes that could be tested. Moreover, it is well settled that the Examiner's assertion that of undue experimentation cannot be based simply on the quantity of experimentation required. As noted in the MPEP § 2164.06, the test for whether experimentation is undue "is not merely quantitative, since a considerable amount of

experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed.' " *In re Wands*, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988).

Rejections under 35 U.S.C. § 102(b) and (e)

The rejection of claims 1-4, 8-10, and 12 for allegedly being anticipated by EP 0902035 A2 and US Patent No. 6,346,379 (referred to collectively herein as Gelfand) is respectfully traversed.

As explained previously, a claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference. *Verdegaal Bros. v. Union Oil Co. of California*, 2 USPQ2d 1051 (Fed. Cir. 1987) and MPEP §2131.

The rejection is based on the assertion that the Gelfand references teach the use of mutant DNA polymerases to incorporate labeled nucleotides into DNA molecules. The Examiner fails to point out, however, where in the experimental sections of these references the template for synthesis is an RNA molecule, as required by the pending claims. Instead the Examiner points to the definition for a DNA synthesis reaction, which is defined to be a method "of producing copies of DNA including but not limited to PCR, strand displacement amplification, transcription mediated amplification, primer extension and reverse transcription." (*see e.g.*, column 7, lines 25- 30). Applicants respectfully submit that this language does not support the Examiner's interpretation. The cited references clearly teach that the enzymes disclosed there can be used in a number of contexts and in many DNA synthesis reactions. The Examiner fails to show, however, that the cited definition explicitly states that each of the activities listed in the definition is carried out by the same enzyme in the reaction mixture. In the absence of such a showing the rejection is improper and should be withdrawn.

Rejection under 35 U.S.C. § 103(a)

The rejection of claims 11, 13-16, 20-32, 36-44, 48-68 over the Gelfand references in view of Kawasaki is respectfully traversed.

As explained previously, to establish a *prima facie* case of obviousness, the Examiner must meet three basic criteria. First, the Examiner must show that there is some suggestion or motivation, either in the cited references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, the Examiner must show a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991) and MPEP § 2142. To support the rejection, the examiner must "present a convincing line of reasoning as to why the artisan would have found the claimed invention to have been obvious in light of the teachings of the references." *Ex parte Clapp*, 227 USPQ 972, 973 (Bd. Pat. App. & Inter. 1985) and MPEP § 2142.

In the present rejection, the Examiner acknowledges that neither of the primary references discloses the use of magnesium, primers and DNA polymerases in reverse transcription reactions of the invention. The teachings of Kawasaki are relied on to provide this missing teaching. As explained in the previous response, those of skill recognized that certain thermoactive DNA polymerases had sufficient reverse transcriptase activity in the presence of manganese, but not magnesium, which was too inefficient to be practical for RT-PCR techniques.

As noted above, a proper rejection under 35 U.S.C. § 103(a) must provide reasoning or evidence to show that one of skill would have a reasonable expectation of success in carrying out the claimed invention. In the present case, the Examiner must provide some reasoning or evidence to show why one of skill would have a reasonable expectation that DNA polymerases of the invention would be sufficiently active in the presence of magnesium. The Examiner points to nothing in the cited references that even remotely suggests that the enzymes taught by Gelfand would be useful in the buffer described in Kawasaki. In the absence of such a showing, the rejection is clearly improper and should be withdrawn.

The Examiner next asserts that relying on unexpected results to support patentability is improper because the claims are not limited to the unexpected results over the

prior art. Applicants respectfully request clarification of this statement since the claims are directed to the use of the enzymes in RT-PCR amplification assays, for example, reactions in which magnesium is present in the reaction mixture. This difference between the claims and the prior art is the basis for the surprising results.

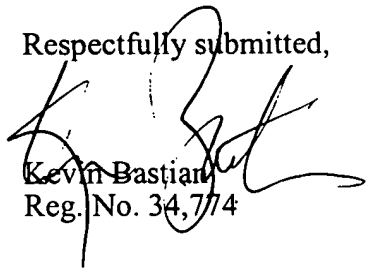
The Examiner also questions applicant's evidence of surprising results because the difference in efficiencies between magnesium and manganese is not clear. These data are not presented to show that magnesium is a ***better*** divalent cation than manganese. Rather the data establish that, despite the expectation that DNA polymerases would be inefficient in the presence of magnesium, the enzymes of the invention were ***as effective*** in magnesium as they are in manganese. This result was not expected.

Finally, the Examiner asserts that the surprising results are not commensurate with the scope of the claims. It is well established that nonobviousness of a genus may be supported by data showing unexpected results of a species if one of ordinary skill in the art would be able to determine a trend in the exemplified data which could be reasonably extended to other members of the genus. MPEP § 716.02 (d) and *In re Kollman*, 595 F.2d 48, 201 USPQ 193 (CCPA 1979). The Examiner has provided no reasoning or evidence to explain why one of skill would doubt the general applicability of the data presented in the present application. As explained in the response enablement rejection above, the relationship between the structure and function of DNA polymerases was well known at the time of the present invention.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested. If a telephone conference would expedite prosecution of this application, the Examiner is invited to telephone the undersigned at 415-576-0200.

Respectfully submitted,



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